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Ciba Foundation Symposium 143

THE BIOLOGY OF HYALURONAN

Exhibit B

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Ref 1

Introduction

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It is a pleasure for me to open this conference on the biology of hyaluronan. The idea of a Ciba Foundation symposium on hyaluronan came after the conference on the functions of the proteoglycans held in 1986 (Ciba Foundation 1986). Hyaluronan was then treated as an 'honorary proteoglycan'. Hyaluronan is, however, a unique polymer, as will be apparent from the discussions during this symposium, and I am glad that the Foundation acted positively on the proposal by Roger Mason and myself to devote a conference entirely to hyaluronan. I want to express our thanks to David Evered and his colleagues for all their efforts in organizing our meeting.

Karl Meyer described a polysaccharide isolated from the vitreous humour in 1934 (Meyer & Palmer 1934). He precipitated the polymer in acid conditions and the product was therefore an acid. It contained uronic acid and Meyer named the polysaccharide *hyaluronic acid* from *hyalos* (= glassy, vitreous) and uronic acid. At physiological pH all carboxyl groups on the uronic acid residues are dissociated and the polysaccharide should therefore be named *sodium hyaluronate* when sodium is the counter ion. It is, however, often difficult to specify the counter ion, for example in a tissue, and Balazs et al (1986) therefore suggested that the name *hyaluronan* should be used, when the polysaccharide is mentioned in general terms. This is in conformity with the accepted terminology that names of polysaccharides should end with -an. Hyaluronic acid and hyaluronate should be reserved to specifically indicate the acid and salt forms of the polymer, respectively.

During the 1930s and 1940s Karl Meyer and others isolated hyaluronan from a number of sources and larger amounts were found, apart from the vitreous body, in other soft connective tissues such as synovial fluid, umbilical cord, skin and rooster comb (see Meyer 1947). The polysaccharide was also isolated from certain strains of bacteria, such as streptococci (Kendall et al 1937). At the same time the hyaluronidases were also described, the first one as a 'spreading factor' in testicular extracts (Duran-Reynals 1942).

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The early chemical characterization of hyaluronan showed that it contained equimolar concentrations of glucuronic acid and *N*-acetylglucosamine. The complete structure was elucidated to a large extent by Karl Meyer and his co-workers in the 1950s. They isolated a crystalline disaccharide, hyalobiuronic acid, from polymer degraded with testicular hyaluronidase and acid hydrolysis (Rapport et al 1951). Structural studies on this disaccharide established the glucuronidic linkage in the polymer. By the use of enzymes and structural analyses on oligosaccharides obtained by enzymic digestion the glucosaminidic linkage could be similarly defined (see e.g. Brimacombe & Webber 1964). The polysaccharide is a linear polymer with the structure . . . (1-[β-4) D-glucuronic acid (1-β-3) *N*-acetyl-D-glucosamine (1-]$_n$-β-4) . . . (see Fig. 1). Subsequent studies on the conformation of the chain by X-ray diffraction and spectroscopy indicate that the molecule can take up helical conformations stabilized by hydrogen bonds. We shall get up-to-date information on the conformational work in John Scott's contribution.

The physicochemical characterization of the polymer was carried out in the 1950s and 1960s. It is notable that Blumberg and Ogston summarized the state of the art at a Ciba Foundation symposium in 1958. Hyaluronan is a linear polymer when visualized in the electron microscope (Fessler & Fessler 1966). It is polydisperse and has usually a weight-average molecular mass of the order of several millions. The chain behaves in solution as an expanded random coil with a diameter of the order of 500 nm. The molecular domain includes a large amount of solvent. The chains entangle already at concentrations in the order of 1 g/l and, as a consequence, at higher concentrations the solutions exhibit an extremely high but shear-dependent viscosity (see e.g. Laurent 1970).

The physical chemistry was used in the 1960s to define possible physiological functions of hyaluronan and other connective tissue polysaccharides (for a review see Comper & Laurent 1978). It was shown that the polysaccharides could regulate the water balance via osmotic pressure and flow resistance; interact with plasma proteins via sieve and exclusion effects; act as lubricants through their rheological properties; and stabilize structures by electrostatic and other interactions.

An important discovery was made by Hardingham and Muir in 1972 when they found that cartilage proteoglycans specifically interact with hyaluronan. Many proteoglycans bind to the same hyaluronan chain and form aggregates which are deposited between the collagen fibres. Hyaluronan thus has a central structural role in cartilage. A large amount of research during the 1970s has been centred on this interaction. Roger Mason will present some aspects of the role of hyaluronan in cartilage in this symposium, and I hope that Dr Hardingham will take an active part in the discussions.

Several developments during the present decade have enhanced our interest in hyaluronan:

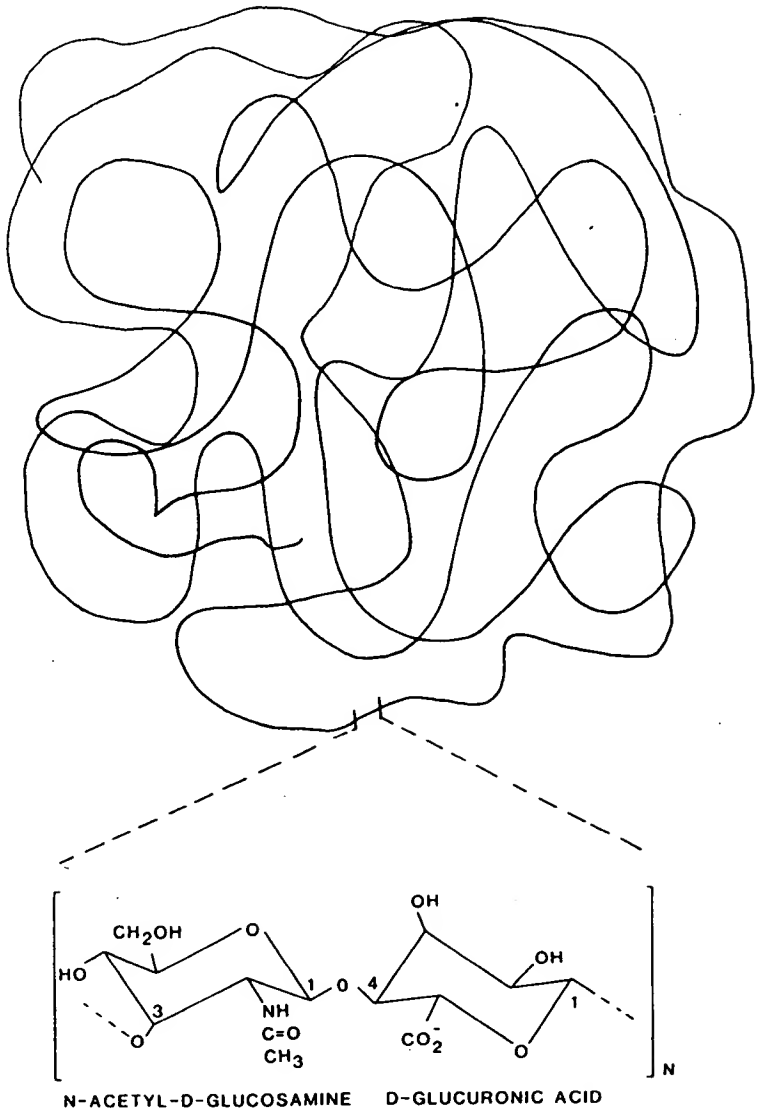

FIG. 1. The basic disaccharide unit of hyaluronan and its expanded random coil formation in solution.

(1) The discovery by Hardingham & Muir (1972) introduced proteins with specific affinity for hyaluronan and these have been used as analytical tools. We can now measure hyaluronan specifically in body fluids with a sensitivity which is 100–1000 times higher than that of previous techniques (Tengblad 1980).

The proteins have also been used to visualize hyaluronan histochemically, and several contributors to this symposium will address themselves to this subject.

(2) Fifty years after the discovery of the polysaccharide we have at last obtained information about the site where and the molecular mechanism by which the polymer is synthesized. We shall probably soon know a great deal more about the regulation of its synthesis. Peter Prehm and Nasi Mian, who have pioneered the field (Prehm 1983, Mian 1986), will introduce us to this fascinating subject.

(3) Similarly, there has been an advance in our knowledge of the turnover and catabolism of hyaluronan, as Robert Fraser and Lennart Rodén will tell us. The elucidation of the catabolic pathways (Fraser et al 1981) has led to interesting clinical applications, of which we shall hear more from Anna Engström-Laurent.

(4) The cell biological role of hyaluronan has been recognized for decades but interest has become focused on this aspect in the last few years. Most of the speakers at this symposium will deal with some type of hyaluronan–cell interaction. (a) There are many reports on the relation between cell growth and hyaluronan synthesis. The polysaccharide may be involved in the mitotic process (Brecht et al 1986). (b) Fibroblasts and other cells surround themselves with a coat of hyaluronan-containing material, which was ingeniously visualized by Clarris & Fraser (1968). The coat may be hyaluronan under synthesis or hyaluronan bound to specific receptors on the cell walls. Charles Underhill and Eva Turley will tell us about hyaluronan-binding proteins, which may act as receptors for hyaluronan and which may also, through this interaction, regulate the cellular functions. (c) The interaction between cells and hyaluronan may be especially important during embryonic and fetal development. Bryan Toole has pioneered the studies on the developmental role of hyaluronan and Bertrand Delpech will discuss its role in the developing brain. (d) Hyaluronan has been assigned interesting functions in for example malignant growth, the immune system, angiogenesis and wound healing. These topics will be covered by Warren Knudson, Theresa Whiteside, David West and Paul Weigel.

(5) Highly viscous hyaluronan preparations have been used as an aid in ophthalmic surgery and one can envisage the exploitation of its rheological properties for other purposes. Endre Balazs, who is the pioneer in the practical use of hyaluronan in what he calls viscosurgery (Balazs 1983), will also contribute to this symposium.

It is my hope that during the symposium we shall be able to shed some light on the biological function of hyaluronan. It is a compound which is ubiquitous and we do not know of any genetic disease in which hyaluronan is not synthesized. This indicates that hyaluronan is of fundamental importance in the animal organism and that mutations causing defects in hyaluronan synthesis are lethal. The participants in this symposium are well qualified

to produce new and original ideas about the functions of this interesting polysaccharide.

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